

Actuation means for the mechanical stimulation of living cells via microelectromechanical systems: a critical review

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Abstract

Within a living body, cells are constantly exposed to various mechanical constraints. As a matter of fact, these mechanical factors play a vital role in the regulation of the cell state. It is widely recognized that cells can sense, react and adapt themselves to mechanical stimulation. However, investigations aimed at studying cell mechanics directly *in vivo* remain elusive. An alternative solution is to study cell mechanics via *in vitro* experiments. Nevertheless, this requires implementing means to mimic the stresses that cells naturally undergo in their physiological environment. In this paper, we survey various microelectromechanical systems (MEMS) dedicated to the mechanical stimulation of living cells. In particular, we focus on their actuation means as well as their inherent capabilities to stimulate a given amount of cells. Thereby, we report actuation means dependent upon the fact they can provide stimulation to a *single* cell, target a maximum of a *hundred* cells, or deal with *thousands* of cells. Intrinsic performances, strengths and limitations are summarized for each type of actuator. We also discuss recent achievements as well as future challenges of cell mechanostimulation.

Keywords: Cell mechanostimulation, cell stretching, cell loading, cell

indentation, microelectromechanical systems (MEMS).

1. Introduction

It is widely recognized that mechanical and biochemical cues occurring at the cellular level prove to be intimately correlated through reciprocal mechanochemical conversion pathways. Indeed, numerous studies have highlighted the fact that surrounding mechanical stresses sensed by a cell may elicit cellular biochemical signals, which in turn may direct and mediate intricate cellular processes. Thereby, externally applied forces may induce profound effects on cellular functions as essential as apoptosis (programmed cell death), growth, proliferation, contractility, migration or differentiation (see Bao and Suresh, 2003; Wang and Thampatty, 2006; Janmey and McCulloch, 2007; Lele et al., 2007; Hoffman and Crocker, 2009 and references therein). This aptitude to modulate cell biochemical reactions constitutes the essence of a very active field of research which might lead to promising applications in biotechnology as well as in medicine. Dysfunctions in mechanotransduction processes contribute to the underlying causes of major diseases including osteoporosis, hypertension, asthma, malaria or cancer (Lee and Lim, 2007). By regulating cellular biochemical reactions via proper mechanical signals, development of pathological conditions might be ideally limited. For instance, one might ultimately envision cell-based therapies wherein mechanical effects on cell fate and growth could affect tissue remodeling and regeneration (Kim et al., 2009a)

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21 Several articles have already reviewed the large panel of experimental tech-
22 niques and microelectromechanical systems (MEMS) reported for conducting
23 mechanobiology studies at the cell level (Van Vliet et al., 2003; Huang et al.,
24 2004; Geitmann, 2006; Addae-Mensah and Wikswo, 2008; Norman et al., 2008;
25 Loh et al., 2009; Sen and Kumar, 2010). These references have usually dis-
26 cussed tools for cell mechanics at a systemic level, whereas very few reports
27 have independently analyzed the actuation or measurement principles involved
28 in these systems. To the best of our knowledge, only Brown proposed a review
29 focused on actuation techniques intending to replicate the different types of me-
30 chanical stresses that cells face *in vivo* (Brown, 2000). Brown discussed systems
31 able to mimic compressive strains (cartilage and bone cells experience compres-
32 sive loads), elongations (lung and heart cells endure stretching cycles during
33 breathing and beating), as well as shear stresses (in blood vessels, cells are con-
34 tinuously subjected to fluid shear stress from blood flow). However, Brown's
35 review focused mainly on early laboratory apparatus which were only able to
36 address large cell cultures or tissues. Meanwhile, recent advances in micro-
37 fabrication techniques have facilitated interactions with isolated cells and more
38 realistic complex cellular environment. Thereby, MEMS appear today as ideal
39 interfaces to integrate more *in vivo*-like stimuli in *in vitro* settings.

40 The aim of this paper is to provide an updated overview of actuation tech-
41 niques dedicated to the mechanical stimulation of living cells via MEMS. In
42 particular, we report initial characterization of principles as a function of their
43 inherent capabilities to target a given amount of cells. Hereafter, sections present
44 various MEMS intended for the stimulation of a single cell, tens of cells (e.g.,
45 maximum 200 cells), and large populations of cells (e.g., minimum 10^4 cells).
46 Finally, discussion of the strengths and limitations for each methodology and a

47 comparative analysis are also included.

48 **2. MEMS for the mechanical stimulation of one cell**

49 This section introduces several MEMS that have been reported in the lit-
50 erature for the mechanical stimulation of a single isolated cell. By definition,
51 the terminology MEMS employed throughout the paper will refer to systems
52 encompassing electrical, optical, or mechanical parts manufactured via micro-
53 fabrication processes. It is however worth noticing that the presence of micro-
54 scopic components is not always a sufficient condition to consider a system as
55 a MEMS. For instance, micropipettes (e.g., Evans and Yeung, 1989; Sato et al.,
56 1990; Miyazaki et al., 2000) or microcantilevers used in atomic force micro-
57 scopes (e.g., Lekka et al., 1999; van der Rijt et al., 2006; Li et al., 2008; Cross
58 et al., 2008; Pillarisetti et al., 2008; Boukallel et al., 2009) are usually considered
59 as experimental tools by the research community (see for instance the classifica-
60 tion adopted in the reviews of Kim et al., 2009a or Loh et al., 2009). Accordingly,
61 and even though they are implicitly considered later in our analysis, they will not
62 be described in details in this paper.

63 In order to avoid too many subcategories in our classification, we also state
64 the following assumptions. Although mechanical stimuli can be applied upon
65 cells either by a controlled force or a controlled displacement, actuation means
66 are reported hereafter independent of the type of physical input. Similarly, no
67 particular distinction is made between systems providing stimulation globally
68 (i.e., stimulation is provided to the entire cell structure) or locally (i.e., only
69 a given cellular region is excited). In addition, we do not differentiate actua-
70 tion means as a function of the type of cells they can target (i.e., adherent or
71 suspended cells). Finally, the consideration of auxiliary equipments (e.g., laser

72 sources, peristaltic pumps, electric power supplies) is out of the scope of this
73 paper.

74 2.1. Electromagnetic fields

Magnetic fields have been used for studying the physical properties of cell cultures for decades (e.g., Crick and Hughes, 1950). However, technological evolutions have been recently reported with the manufacturing of microscopic magnetic manipulators able to locally stress an isolated cell (e.g., Chiou et al., 2006; Kanger et al., 2008; Yapici et al., 2008). For instance, in (de Vries et al., 2004, 2005), the authors implemented three magnetic micropoles on a glass substrate (see Fig. 1) in order to enable the stimulation of one cell in two dimensions. Each pole tip was $4\text{ }\mu\text{m}$ wide, $6\text{ }\mu\text{m}$ thick and had a surface roughness of $0.5\text{ }\mu\text{m}$. Poles spacing was about $20\text{ }\mu\text{m}$ to ensure the placement of a single cell between them. To transfer mechanical stimuli, magnetic microspheres were functionalized (i.e. coated with biochemicals) to allow their binding to specific cellular receptors. Once anchored, such microbeads could act as *handles*. Indeed, in the presence of a spatially varying magnetic field, the force F_{mag} experienced by such a magnetic particle is:

$$F_{mag} = \nabla(m \cdot B) \quad (1)$$

where m is the magnetic moment of the microparticle and B is the magnetic flux density. Assuming the induced moment is parallel to the magnetic field, and the field is large enough such that the magnetization of the particle saturates, the force acting on the magnetic particle can be approximated by the equation:

$$F_{mag} = MV \frac{dB}{dx} \quad (2)$$

75 where M and V are the magnetization and the volume of the particle, respec-
 76 tively. Thereby, by controlling the amplitude and the direction of the magnetic
 77 flux gradient generated at the center of the three micropoles, de Vries et al. ex-
 78 perimentally validated actuation forces up to 12 pN on magnetic microbeads of
 350 nm diameter.

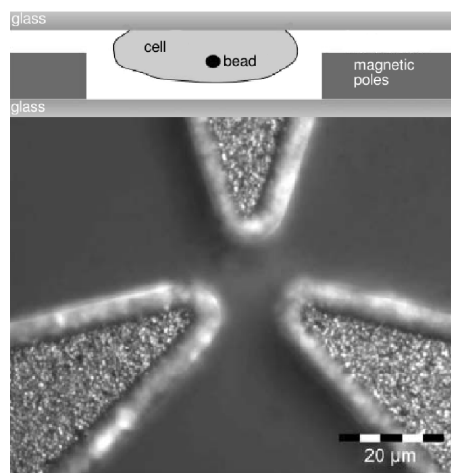


Figure 1: Top: sketch illustrating the setup designed by de Vries et al.: a cell anchored to a glass plate and embedding a magnetic microbead is placed between the tips of magnetic poles. Bottom: microscope image showing the extremities of three magnetic micropoles. Images adapted from (de Vries et al., 2005)

79

80 2.2. Microactuators generating electric fields

81 Non-uniform electric fields offer an alternative option to physically deform
 82 an isolated cell (e.g., Engelhardt and Sackmann, 1988; Wong et al., 2005; Riske
 83 and Dimova, 2006; Dimova et al., 2007; Guido et al., 2010; MacQueen et al.,
 84 2010). Indeed, when a cell is subjected to an electric field, a dipole can be in-
 85 duced due to interfacial polarization on the cell membrane. Depending on the
 86 electric field strength and the effective polarization of the cell, stress can then

87 occur at the interfaces and result in a deforming force. During minor deforma-
 88 tion, the elastic strain of the cell along the electric field direction is estimated as
 89 (Sukhorukov et al., 1998):

$$\frac{\Delta L_C}{L_{C0}} = K_S E^2 \text{Re}[U(\omega)] \quad (3)$$

90 where ΔL_C represents the deformation of the cell, L_{C0} is the original length of
 91 the cell, K_S is a constant representing the elastic properties of the cell, ω is the
 92 angular frequency of the AC electric field applied, and $U(\omega)$ is the complex
 93 Clausius-Mossotti factor that depends on the internal structures of the cell and is
 94 cell-type specific.

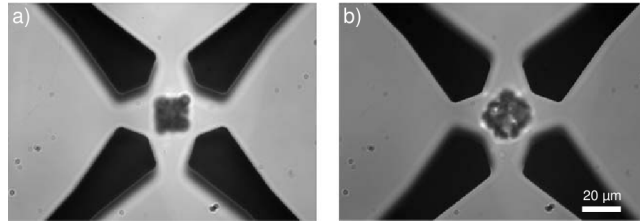


Figure 2: a) A GUV trapped between the electrodes of a microfield cage. b) The GUV is deformed by electric field. Images adapted from (Korlach et al., 2005)

95 Illustration of an octode microfield cage able to capture, hold, rotate and de-
 96 form isolated giant unilamellar vesicles (GUVs) is given in Fig. 2 (Korlach et al.,
 97 2005). Modulation of the amplitude and frequency of the voltage applied to the
 98 electrode edges permitted the authors to conduct stretch and relax experiments
 99 on isolated GUVs, whose size ranged from 5 to 25 μm .

100 2.3. Microactuators based on optical gradients

101 Both refraction and reflection of light exert forces on all objects. If these
 102 forces are negligible in the macroworld, they become significant for microscopic

103 objects weighing less than $1\text{ }\mu\text{g}$. Thereby, light has been used to manipulate
 104 microparticles for four decades (e.g., Ashkin, 1970). Two optical fibers can be
 105 used to guide the light emanating from a laser source and create a dual beam
 106 laser trap system (e.g., Constable et al., 1993; Singer et al., 2003). In (Guck
 107 et al., 2001, 2002), the authors made use of optical fibers with a diameter of
 108 $125\text{ }\mu\text{m}$ to trap and stretch biological entities. The divergent laser beams were
 109 directed at diametrically opposite portions of a suspended cell placed between
 110 them, as shown in Fig. 3. Often termed as optical stretcher (OS) in the literature,
 111 the net stretching force F_{os} exerted by such a configuration on a single cell can
 112 be expressed by the following equation (Van Vliet et al., 2003):

$$F_{os} = \left(n_m - (1 - R) n_c + R n_m \right) \left(\frac{P}{c} \right) + \left(n_c - (1 - R) n_m + R n_c \right) \left((1 - R) \frac{P}{c} \right) \quad (4)$$

113 where n_m and n_c are the refractive indices of the surrounding media and cell,
 114 respectively, R is the fraction of reflected light, c is the speed of light in vacuum,
 115 and P is the total light power. With a 500 mW power laser source, this approach
 116 allowed Guck and co-workers to generate uniaxial stretching forces up to 400
 117 pN in aqueous media. This facilitated cell elongations between $7\text{-}30\text{ }\mu\text{m}$. Guck
 118 et al. even predicted that given a higher power laser, the maximum stretching
 119 force could achieve or exceed 1 nN.

120 2.4. Electrothermal microactuators

121 Thermal expansion caused by electric currents heating up the material of a
 122 microstructure constitutes another well known actuation principle used in MEMS

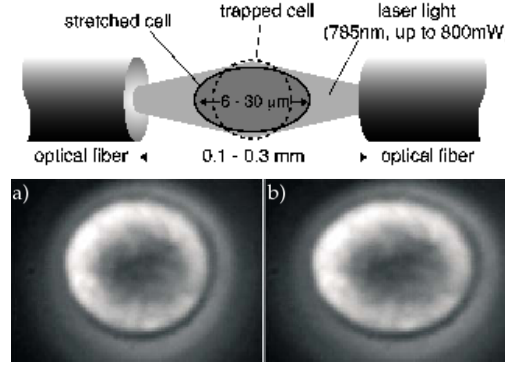


Figure 3: Top: representation of the all-fiber OS put forward by Guck et al. Bottom: a red blood cell, approximately $10 \mu\text{m}$ in diameter, trapped by OS: before (a) and during (b) stretching (Guck et al., 2001, 2002)

(e.g., Zhu et al., 2006; Lu et al., 2006; Espinosa et al., 2007; Christopher et al., 2010). In particular, large rectilinear displacement parallel to the device substrate can be achieved with *chevron* (or V-shaped beam) configurations. Such a compliant beam is depicted in Fig. 4.

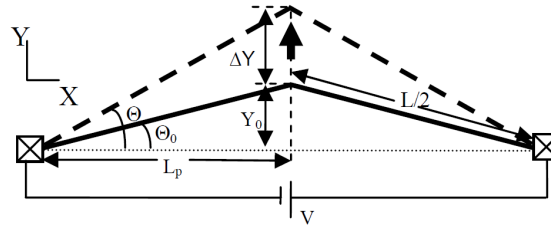


Figure 4: Main dimensions of a V-shaped beam (or *chevron*) anchored at its two ends: Joules heating causes thermal expansion and pushes the apex outward when an electric current passes through the structure (Kushkiev and Jupina, 2005)

126

Displacement of the beam apex ΔY can be approximated via the formula (Girbau et al., 2003):

$$\Delta Y = \left(\frac{L + \Delta L}{2} \right) \sin \left[\arccos \left(\frac{2L_p}{L + \Delta L} \right) \right] - Y_0 \quad (5)$$

where L is the total beam length, L_p is the X axis projection of $L/2$, and Δ_L is the increment in length of the beam which can be expressed by:

$$\Delta_L = \frac{\alpha q L^3}{12k} \quad (6)$$

In equation 6, α is the thermal expansion coefficient, k is the thermal conductivity, $q = V^2/(LwtR)$ is the heat generation per unit volume, V is the voltage applied between anchors, whereas w , t , R are the width, thickness and electrical resistance of the beam, respectively. Multiple pairs of such V-shaped beams can be serially combined in order to reach higher force displacement. Indeed, for small displacement, the total actuation force of several V-shaped beams can be approximated by:

$$F_{therm} = N \frac{E w^3 t}{4 L^3} \Delta Y \quad (7)$$

127 where E is the Young's modulus and N is the number of beams.

128 Compression of a mouse fibroblast (NIH3T3) with an array of five *chevrons*
 129 has been reported by Zhang et al. (2008) (see Fig. 5). This miniature cell loading
 130 system was power supplied either by low continuous voltages (≤ 2 V) when
 131 operating in air, or by high frequency (800 kHz) sinusoidal voltages in liquids.
 132 In ambient conditions, it offered a maximum translation along one direction of
 133 $9 \mu\text{m}$. This MEMS allowed the authors to apply compressive strains up to 25%
 134 of the initial cell size.

135 2.5. Electrostatic microactuators

Many MEMS intended to the fatigue investigation of micro and nanomaterials have been actuated by interdigitated comb fingers exploiting electrostatic phenomena (e.g., Kahn et al., 1999; Kiuchi et al., 2007; Naraghi and Chasiotis, 2009; Takahashi et al., 2009). Biological applications have been reported by

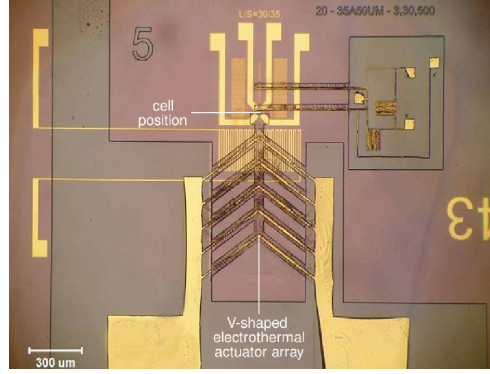


Figure 5: Electrothermal MEMS cell loader designed for measuring the compliance of cells. Image adapted from (Zhang et al., 2008)

Eppell et al. (2006) and Shen et al. (2008), who carried out stress-strain experiments on individual collagen fibrils. A multidimensional approach based on a single linear electrostatic structure was also reported by Scuor et al. (2006), who conceived a micro in-plane biaxial cell stretcher (see Fig. 6). The quadrants of a sliced circular plate were actuated in mutually-orthogonal directions, that is to say that the quadrants moved in horizontal and vertical directions simultaneously. The net force developed by such a comb drive actuator is given by:

$$F_{electro} = N \left(\frac{\epsilon t}{g} \right) V^2 \quad (8)$$

136 where N is the number of comb electrodes, ϵ is the permittivity constant of the
 137 dielectric medium, t is the comb thickness, g is the comb electrode gap and V is
 138 the driving voltage. Theoretically, Scuor et al. claimed that a nominal voltage of
 139 100 V permitted such an electrostatic structure to generate actuation forces up
 140 to $60 \mu\text{N}$. In practice, only translation amplitudes of the plate were reported. In
 141 ambient conditions, a power supply of 100 V led to a maximum space between
 142 the quadrants of $3.4 \mu\text{m}$.

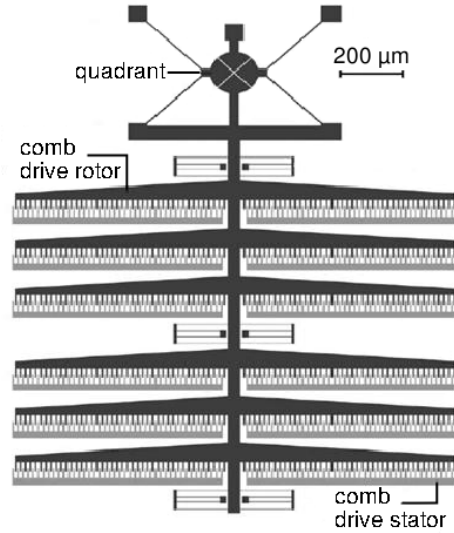


Figure 6: Illustration a comb drive system actuating a bi-axial cell stretcher. Drawing adapted from (Scuor et al., 2006)

2.6. Micro-nanopositioning stages

Commercial micro or nanopositioning stages (or micro-nanotranslators) may be classified as *off-chip* actuators. Unlike the actuation means presented so far, they are distantly linked to the microstructure they control (see Fig. 10 for an illustration). It is worth noticing that the prefix *micro-nano* often encountered in the literature is not related the size of these actuators, but to their displacement resolution. However, they are one of the most widespread option for ensuring the actuation of passive MEMS dedicated to the stimulation of cells. Thereby, positioning stages are conventionally used to actuate passive microstructures such as microplates (e.g., Thoumine et al., 1999; Desprat et al., 2006; Fernández et al., 2006; Gladilin et al., 2007; Chan et al., 2008) or microindenters (e.g., Koay et al., 2003; Peeters et al., 2005; Sato et al., 2007).

Similarly, positioning stages were used by Yang and Saif (2005, 2006, 2009)

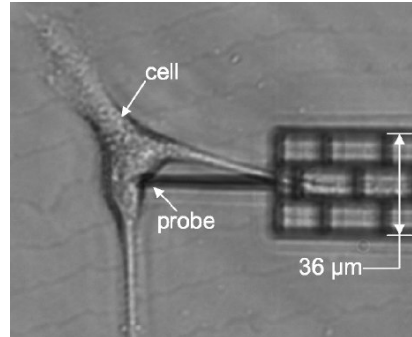


Figure 7: Microscope image of an adherent MKF indented in three dimensions by a force sensor (Yang and Saif, 2005)

to translate compliant microstructures. The extremity of such MEMS is shown in Fig.7. Piezoelectric stages offering an intrinsic resolution of 1 nm were selected in order to apply large strains to adherent fibroblasts in three dimensions. However, these stages were subsequently mounted on a x-y-z mechanical station which lowered the resolution to 1 μm. During experiments, monkey kidney fibroblasts (MKFs) could be indifferently subjected to indentation or stretching with amplitude as large as 50 μm, which was about twice the initial size of the cells.

An off-chip piezoelectric stage was also required to actuate the MEMS-based cell puller of Serrell et al. (2007, 2008). Fig. 8 shows the microfabricated structure which was based on a circular platform split in two parts, one of them being movable. The latter, which was linked to the piezoelectric stage, could be translated along one direction with maximum travel range of 50 μm, a displacement resolution of 0.4 nm and a bandwidth of 520 Hz.

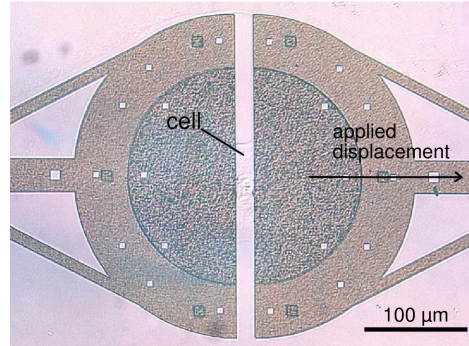


Figure 8: Close-up of a MEMS-based tensometer: an adherent cell anchored in the middle of a disk can be stretched via the translation of a movable part. Image adapted from (Serrell et al., 2007)

170 3. MEMS for the mechanical stimulation of a small group of cells

171 Rather than conduct experiments by repetitively stressing single cells one
 172 after the other, several studies have tried to speed up cell stimulation by targeting
 173 a larger number of cells *concurrently*. To this end, several research teams have
 174 extended concepts initially intended for the stimulation of individual cells by
 175 duplicating given patterns.

176 3.1. Parallelized stimulation with an array of electromagnetic microactuators

In Sniadecki et al. (2007, 2008), the authors fabricated, characterized and tested a dense bed of soft micropillars arranged in a pattern array. Spatial resolution of the array was $9\mu\text{m}$, whereas each pillar measured $1.5\mu\text{m}$ in radius, $10\mu\text{m}$ in height and had a low stiffness of $32\text{ nN}/\mu\text{m}$. With such dimensions, the investigators were able to provide local stimulation to adherent cells lying on the surface of the micropillars through the use of a horizontal uniform magnetic field. The latter was generated by external NdFeB magnets which controlled the bending of certain pillars (see Fig. 9). Indeed, magnetic cobalt nanowires

(350 nm in diameter, 5-7 μm long) were incorporated within some pillars during the fabrication process of the array (1 nanowire per 200 pillars). Attracted magnetic wires enabled the bending of the magnetized pillars up to 15° relative to the pillars' longitudinal axis. Such bending led to a pillar displacement ranging from 100 nm to 1 μm . For a cell positioned at the top of a magnetic pillar, this displacement transferred a punctual force to the focal adhesion sites of the cell. The magnitude of this force was a function of the pillar as well as the nanowire dimensions, in accordance with the following equation:

$$F_{Mag} = \frac{3\mu_{\perp} B(L + L_W)}{2(L^2 + L_W L + L_W^2)} \quad (9)$$

177 where L and L_W are the lengths of the post and the length of the embedded
 178 nanowire respectively, and μ_{\perp} is the component of the dipole moment perpendicular to the magnetic field B , as represented in the inset c) of Fig. 9.

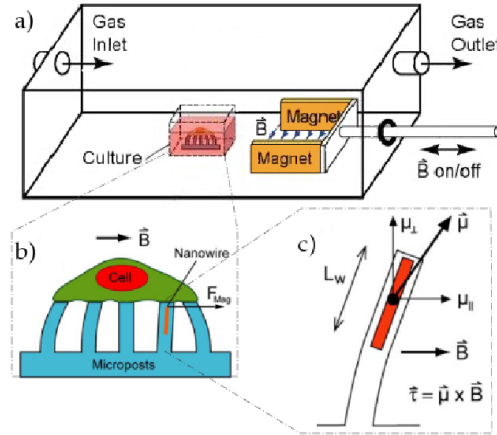


Figure 9: a) Experimental setup of Sniadecki et al. where permanent magnets generate a magnetic field surrounding a cell culture chamber. b) Close-up of the cell culture chamber: an adherent cell is lying on a bed of micropillars, one of them incorporating a magnetic nanowire. c) Parameters influencing the bending of a magnetic pillar in accordance with Equation (9). Drawings adapted from (Sniadecki et al., 2007, 2008)

179

180 For a nanowire of length $L_w=5\mu\text{m}$, a magnetic field B of 0.31 T created
181 a torque of 210 nN/ μm . During experiments, a maximum force of 27 nN was
182 validated by the authors. One may note that this work was originally intended
183 for the local stimulation and study of individual mouse fibroblasts. However,
184 and considering the simple structure adopted by the authors, we believe that
185 such system could be further extended, and readily transposed to the stimulation
186 of tens of cells.

187 *3.2. Parallelized stimulation with an array of microbeams actuated by positioning*
188 *stages*

189 Sasoglu et al. (2007, 2008) manufactured a comparable array of compliant
190 microposts for stretching axons of multiple neurons aligned in a regular pattern.
191 Pillars were however larger, with a diameter of $40\mu\text{m}$, a length of $120\mu\text{m}$. The
192 separation at the base of the pillars was also wider. As opposed to the device
193 proposed by Sniadecki et al., this array was not intended to offer subcellular
194 spatial resolution. Instead, each cell was attached to the free end of a pillar
195 and could be entirely stretched. To control the bending of the micropillars, the
196 authors favored a distant micromanipulation station (see Fig. 10) which offered
197 a precision of 40 nm. With this configuration, the authors claimed that tensile
198 forces as small as 250 +/- 50 nN and as great as 25 +/- 2.5 μN could be exerted
199 on the specimens under investigation.

200 *3.3. Parallelized stimulation with an array of Electro-Active Polymer (EAP)*
201 *microactuators*

202 EAP are polymers that change in shape or size in response to an electrical
203 stimulation. An array of $100\times 100\mu\text{m}^2$ EAP microactuators was built by Ak-

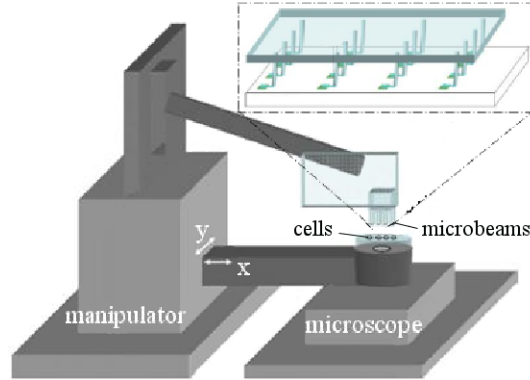


Figure 10: Concept of a micropost array where axons of tens of neurons can be stretched in parallel via the translation of a distant micropositioning stage. Drawings adapted from (Sasoglu et al., 2007, 2008)

204 bari et al. (2010) to perform the individual stretching of 128 cells. In this array
 205 (see Fig. 11), compliant gold electrodes ($100\mu\text{m}$ wide) were deposited by low
 206 energy ion implantation on each side of a $30\mu\text{m}$ thick, 30% pre-stretched, PDMS
 207 (polydimethylsiloxane) membrane. Next, the membrane was placed over a rigid
 208 PDMS support composed of $200\mu\text{m}$ wide channels. The membrane provided
 209 flexibility and could expand over the channels when high voltages were applied
 210 to the electrodes. This design permitted to restrict the stimulation areas to in-
 211 tersections between electrodes and channels. Although this technique was not
 212 applied to living cells, the investigators predict that each cell could potentially
 213 receive up to 10-20% uniaxial strains.

214 4. MEMS for the mechanical stimulation of a large cell population

215 The possibility to stimulate larger cell samples may be seen as a logical next
 216 step. In this section, we arbitrarily define that the actuation principles described
 217 hereafter can deal with a cell population including at least *thousands of cells*. The

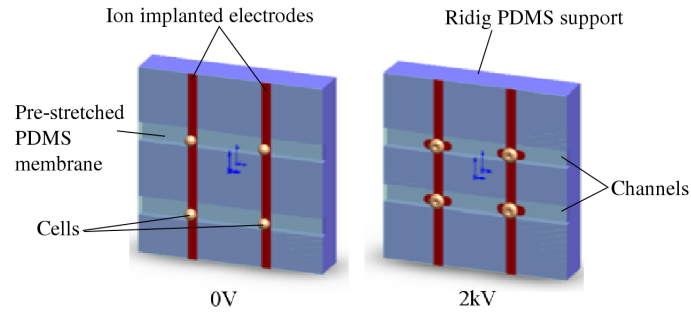


Figure 11: Concept of an array of EAP microactuators. Left: device at 0V with four cells placed at the intersection between electrodes and channels. Right: device when high voltage (2kV) is applied; the four cells are stretched along the channels. Drawings adapted from (Akbari et al., 2010)

only objective of this minimum is to ensure a sufficient difference in the number
of cells to be stimulated in order to guarantee that such large samples cannot be
addressed by the limited throughput configurations presented in Section 3.

4.1. Simultaneous stimulation

Hereafter, we introduce some MEMS able to inherently stimulate very large
amounts of cells concurrently. In order to do this, such MEMS directly stress
entire cell populations.

4.1.1. Simultaneous stimulation with cell substrate deformation

Laboratory devices for the stretching of tissues or large cell populations cul-
tured on thin compliant substrates served as initial tools to investigate the effects
of mechanical cues on living cells (e.g., Norton et al., 1995; Sotoudeh et al.,
1998; Clark et al., 2001; Pfister et al., 2003). This concept can be scaled down
to the microscale level, and MEMS devoted to the distention of cell substrates
have been actuated by electrostatic actuators (Wu et al., 2005), fluids (Kim et al.,
2007), and air pressure (Sim et al., 2007; Tan et al., 2008; Moraes et al., 2010).

233 In (Kamotani et al., 2008), the authors designed a refreshable Braille display
 234 to individually bend up to 24 deformable microwells (see Fig. 12). Each
 235 well measured 1.7 mm in diameter, and the bottom was constituted of a PDMS
 236 membrane, with a Young's modulus approximately 750 kPa, a Poisson ratio's of
 237 0.49, and a thickness ranging between 100-200 μm . Cells to be stressed were
 238 directly cultured on the PDMS membranes, and the pins of the Braille display
 239 were piezoelectrically actuated. The frequency and duration of the stretching ap-
 240 plied by each pin could be controlled via a computer. Maximum extension of the
 241 pins decreased from 0.7 mm for no load to 0.3 mm when the pins pushed a mem-
 242 brane 200 μm thick. A pushing force of 0.18 N was experimentally validated by
 the authors.

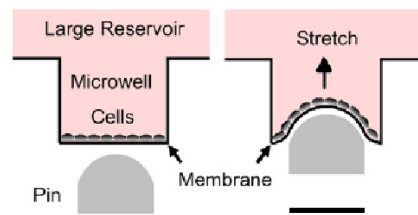


Figure 12: Bending and stretching of soft microwells via Braille display pins. Scale bar represents 1.25mm (Kamotani et al., 2008)

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244 4.1.2. *Simultaneous stimulation with fluid flows*

245 At the macroscale, experimental apparatus such as cone-and-plate rotating
 246 chambers (e.g., Furukawa et al., 2001) or parallel-plate flow channels (e.g., Dong
 247 and Lei, 2000) are conventional tools to impose hydrodynamic shear-stress on
 248 large cell cultures. With advances in microfabrication technologies, microscopic
 249 parallel-plate channels have been reported (e.g., Song et al., 2005; Young et al.,
 250 2007; Tkachenko et al., 2009). In (Lu et al., 2004), the authors integrated four

parallel-plate channels of different cross-sections on a single miniature fluidic
chip. Channel height was $25\ \mu\text{m}$ whereas channel width ranged from $250\ \mu\text{m}$ to
 $1000\ \mu\text{m}$. Such small dimensions guaranteed a low Reynolds number ($\text{Re} \leq 1.0$),
ensuring a laminar flow with no turbulence within the microchannels.

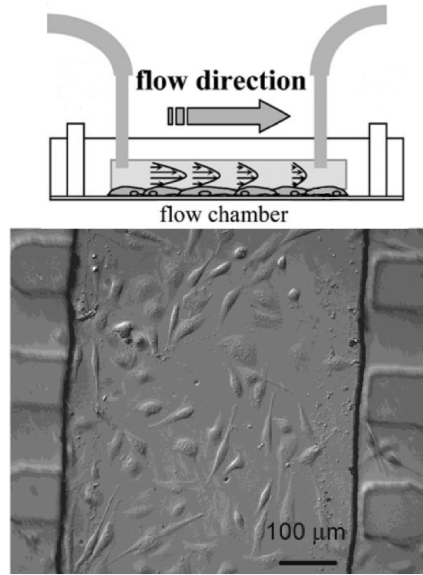


Figure 13: Top: sketch representing the principle of a microfluidic channel imposing shear stress to a culture of adherent cells (Tsou et al., 2008). Bottom: microscope view of fibroblasts cultured in one of a parallel-plate flow chambers. Average fibroblast diameter was about $20\ \mu\text{m}$ after attachment (Lu et al., 2004)

For a parallel-plate channel with an infinite aspect ratio, the generated wall shear stress can be expressed as:

$$\tau_w = \left(\frac{6\mu}{h^2 w} \right) Q \quad (10)$$

where μ denotes the fluid viscosity, h and w are the height and the width of the chamber, respectively, and Q is the volumetric flow rate. Therefore, by varying the width of the channels, Lu et al. could expose a culture of fibroblasts to

multiple shear stress conditions. This allowed the authors to mimic a variety of stresses that vascular cells naturally undergo in the vessel architecture of the arterial system. During experiments, shear stresses up to 4000 dyne/cm^2 were generated by the authors.

4.2. *Serial approaches for high throughput stimulation*

Serial approaches constitute an alternative option to stimulate thousands of cells. Hereafter, we introduce some MEMS able to stress isolated cells sequentially at high stimulation rates.

4.2.1. *Serial stimulation with constricted channels*

If fluids can naturally expose cells to shearing stresses, they can also be used to transport suspended cells toward excitation areas. In (Brody et al., 1995; Youn et al., 2008; Hou et al., 2009), suspended cells were serially guided toward synthetic lattices of constricted areas. This approach allowed Kim et al. (2009b) to mimic the segmental contractions undergone by bovine embryos in a oviduct. As shown in Fig. 14, compressive stresses occurred while the embryos traveled through the constricted areas (i.e., circular channels incorporating areas with a smaller inner diameter). For embryos with a diameter ranging approximately from 150 to $190 \mu\text{m}$, the authors reported compressive forces up to $0.8 \mu\text{N}$.

4.2.2. *Serial stimulation with optical stretchers (OS) and electric fields*

In (Lai et al., 2008; Remmerbach et al., 2009; Lautenschläger et al., 2009), OS similar the one depicted in Fig. 3 were combined with microchannels. Fluid flows ensured the continuous and fast delivery of suspended cells toward the divergent laser beams emanating from the two optical fibers. Thereby, flowing cells could be trapped one by one. Variations of light intensity then allowed the

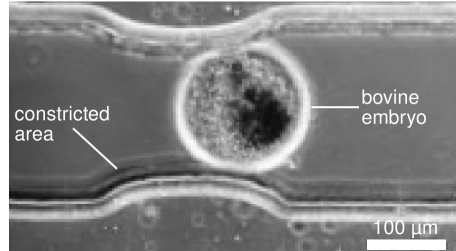


Figure 14: Microfluidic channel including a constrictive area. The image shows a bovine embryo being compressed while crossing the narrow section of the channel. Image adapted from (Kim et al., 2009b)

modulation of the amount of stretching applied to the trapped cell. In particular, stimulation rate up to 100 cells/hour was reported with such an approach (Lincoln et al., 2007).

Similarly, microchannels have been associated with surrounding electric fields. In (Bao et al., 2008), electric field intensity was concentrated toward the narrow section of a microchannel (see Fig. 15). During experiments, field intensities of 200 V/cm, 400 V/cm as well as 600 V/cm were applied. Stress indirectly arose from the electroporation phenomena. In effect, cells may open up pores when they experience an external electric field with an intensity beyond a certain threshold. Material exchange across the membrane may then occur. A direct consequence was the swelling of human breast epithelial cells while they were flowing through the microchannel. Even though the amount of stress induced was not explicitly quantified by the authors, such method allowed to strain suspended cells at stimulation rates as high as 5 cells/s.

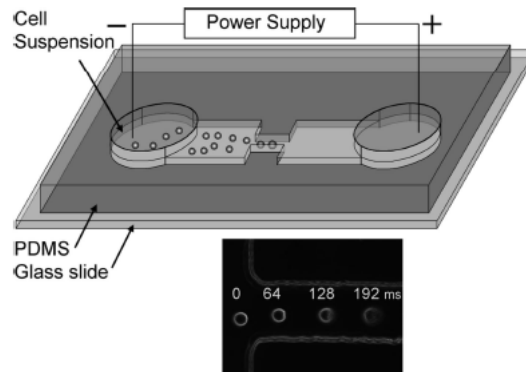


Figure 15: Top: electric fields used in conjunction with microfluidic channel to provoke cell electroporation. Inset: swelling evolution at different times for a cell experiencing electroporation while progressing through a microchannel (Bao et al., 2008)

5. Mechanical stimulation of cells: discussion about the number of cells targeted

On the basis of the details presented in the previous sections, the following questions might be legitimately asked: why aim to stimulating more than one cell? What are the differences between actuation systems targeting tens of cells and those targeting thousands of cells? Are the latter better simply because they can deal with a larger amount of cells? As a matter of fact, the answers to these questions are rather complex. Indeed, in the specific context of cell mechanostimulation, engineering specifications become intercorrelated to biological factors. Hereafter, we discuss some parts of the answers.

5.1. Mechanical stimulation of a single cell: strengths and weaknesses

The large variety of actuation methods that were summarized in Section 2 demonstrates that the stimulation of a single isolated cell has been largely addressed. Indeed, for different but complementary reasons, both life sciences and

312 engineering communities have been highly involved in the development of sys-
313 tems able to interact with a single cell. Recent achievements in this enterprise
314 have marked a milestone in cell mechanics. The possibility to interact with an
315 individual cell has enabled tremendous breakthroughs by helping cell biologists
316 to elucidate how a cell receives and processes extracellular mechanical signals.

317 A major advantage attributed to almost all devices of Section 2 is that both
318 localization and magnitude of the stress applied upon a cell can be finely tuned.
319 This is certainly a necessary condition to conduct successful experiments on liv-
320 ing cells. On the other hand, one may highlight the fact that in most works cited,
321 delicate and time-consuming steps are often required to properly place the cell
322 prior to stimulation. For instance, in (Eppell et al., 2006; Shen et al., 2008),
323 the authors used small drops of epoxy to attach a fibril between the two pads of
324 their uniaxial cell tenser. It is reasonable to assume that such "gluing" chemicals
325 may interact with the living cell, having a certain impact on the intrinsic cell
326 mechanical properties.

327 It is important to note that cells are often considered as passive and ho-
328 mogeneous viscoelastic materials. In effect, such assumptions greatly simplify
329 the modeling of living cells (Lim et al., 2006). In actuality, cells are highly
330 anisotropic entities whose mechanical properties can evolve both in time and
331 space over a variation of several orders of magnitude. Thereby, it has been ex-
332 perimentally observed that an identical mechanostimulus may actually engender
333 variable cell mechanical responses from cell to cell, even within a given cell
334 line. A more representative overview of the cellular behavior could be obtained
335 by considering the averaged responses of many individual cells subjected to the
336 same mechanical stress. A new tendency based on statistical studies has hence
337 progressively emerged (see for instance Mizutani et al., 2008, Hiratsuka et al.,

2009). Unfortunately, MEMS of Section 2 do not ideally lend themselves to the fast stimulation of many cells since they usually involve long protocols aimed at properly preparing the cell prior to experiment (e.g., ensuring a sufficient attachment of the cell on functionalized probes), the stimulation of just a few cells may still take several hours.

5.2. Mechanical stimulation of tens of cells: strengths and weaknesses

To increase cell stimulation rate, arrays of microactuators have been developed to stimulate small groups of isolated cells, as seen in Section 3. Via the duplication of structures (e.g., microposts, microcantilevers) originally intended for the stimulation of an isolated cell, these devices try to preserve the initial advantages of single actuators. It is however worth noting that if individual access to each cell remains possible, actuators are usually not individually controlled. Although the possibility to independently control several groups of EAP actuators has been recently reported in (Akbari et al., 2010), the ability to individually tune the magnitude and localization of the stress applied upon each cell is often partly lost. However, the real shortcoming of these array configurations is relative to their lack of *scalability*.

Indeed, the duplication of perfectly identical structures at the microscale remains limited to a certain extent. Indeed, the fabrication of an array which would include thousands of microactuators still poses formidable challenges. This is representative of a *technological gap*. This limit is represented in Fig. 16, which also illustrates the fact that, in addition to technical complexity, large replication of patterns will usually induce a significant increase in cost. Thereby, and to the best of our knowledge, no array configuration can presently stimulate thousands

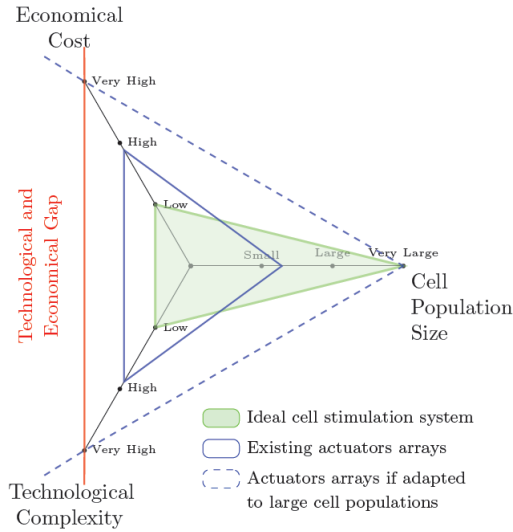


Figure 16: Prediction highlighting the limitation of current microactuators arrays (blue triangle): they cannot be easily transposed to the stimulation of very large cell populations. In contrast with an ideal stimulation system (green area), we indeed foresee that even the replication of simple repetitive patterns could not be indefinitely extended without drastic increase in both cost and technological complexity (dashed lines).

of isolated cells. Meanwhile, studies conducted on tens of cells may still appear as modest populations compared to the colossal number of cells that constitute a living organism.

5.3. Mechanical stimulation of thousands of cells: strengths and weaknesses

Alternative configurations targeting thousands of cells have also been developed. As presented in Section 4, the culture of a large population of *adherent* cells on a thin compliant substrate (see Fig. 12) can facilitate the transfer of mechanical stress to the whole cell population by simply distorting the substrate. While this approach permits the stimulation of a very large number of cells in a simple manner, several restrictions apply. Generally speaking, and independent

374 of the type of actuator used to induce substrate deformation, stress distribution
375 remains usually inhomogeneous. Indeed, depending on the Poisson's ratio of
376 the material used, even if one stretches (or bends) the thin substrate solely along
377 one dimension, coupling between radial and tangential strains occurs during sub-
378 strate distention. Therefore, based on their position on the substrate, all cells are
379 not subjected to the same amount of stress. Most importantly and unlike the ma-
380 trix configuration of Section 3, the individual stimulation of a particular cell is
381 completely lost.

382 To mitigate the latter restriction, configurations involving microchannels with
383 fluid flows that allow the serial delivery of individual suspended cells toward ex-
384 citation areas have also been explored. In particular, when coupled to laser beams
385 or external electric fields, microfluidic chips such as the one of Fig. 15 offer the
386 possibility to modulate the stress intensity applied upon each cell while also
387 achieving relatively high stimulation rates. This paved the way for microsystems
388 aimed at offering high throughput cell stimulation. Despite these remarkable ad-
389 vantages, these configurations work exclusively with suspended cells showing
390 high degree of symmetry and/or high optical uniformity. Unfortunately, this ex-
391 cludes studies of adherent cells.

392

393 **6. Actuation means of MEMS for the mechanical stimulation of cells: com-** 394 **parative analysis**

395 It is now clear that a large number of different actuation means are available
396 for the mechanical stimulation of living cells. Among this wide variety, one
397 might wonder if a *ranking* could be established comparing these technologies.
398 In other words, is one actuation principle better than another? Which actuation

399 type should be used in the design of a new MEMS intended to apply mechanical
400 stimuli upon cells? In this section, we discuss some of relevant aspects of cell
401 mechanostimulation that make it such a complex and delicate task.

402 6.1. Notion of stress control

403 During the mechanical excitation of living cells, an optimal actuation mean
404 should offer a high degree of accuracy in the control of the physical constraint
405 applied. Ultimately, it is critical to mimic the constraints faced by cells *in vivo*.
406 Moreover, it is vital to avoid the generation of stress with improper orders of
407 magnitude that could cause irreversible damages to living cells. The chart from
408 Fig. 17 gives an overview of the inherent performances for each type of actuation
409 mean and relates their respective resolutions both in terms of displacement and
410 force.

411 Additionally, the orders of magnitude in the chart have been scaled accord-
412 ing to relevant information and data collected from various sources. Therefore,
413 our set of actuation techniques were not based purely on a restricted number of
414 particular MEMS. For instance, performances of positioning stages have been
415 evaluated based on the large panel of product references and datasheets avail-
416 able from manufacturers such as *Physik Instrumente* (PI). Likewise, lower and
417 upper bounds fixing global performances of piezoelectric, electrostatic, as well
418 as electrothermal microactuators have been extrapolated from (Bell et al., 2005;
419 Hubbard et al., 2006; Naraghi et al., 2010). In order to accurately characterize
420 the overall capabilities of each actuation technique, it is also essential to consider
421 several cell studies conducted via experimental configurations. For air pressure,
422 data have been extracted from (Hochmuth et al., 1993; Hochmuth, 2000; Chu
423 et al., 2004; Sanchez et al., 2008). Values for fluid flows have been based on

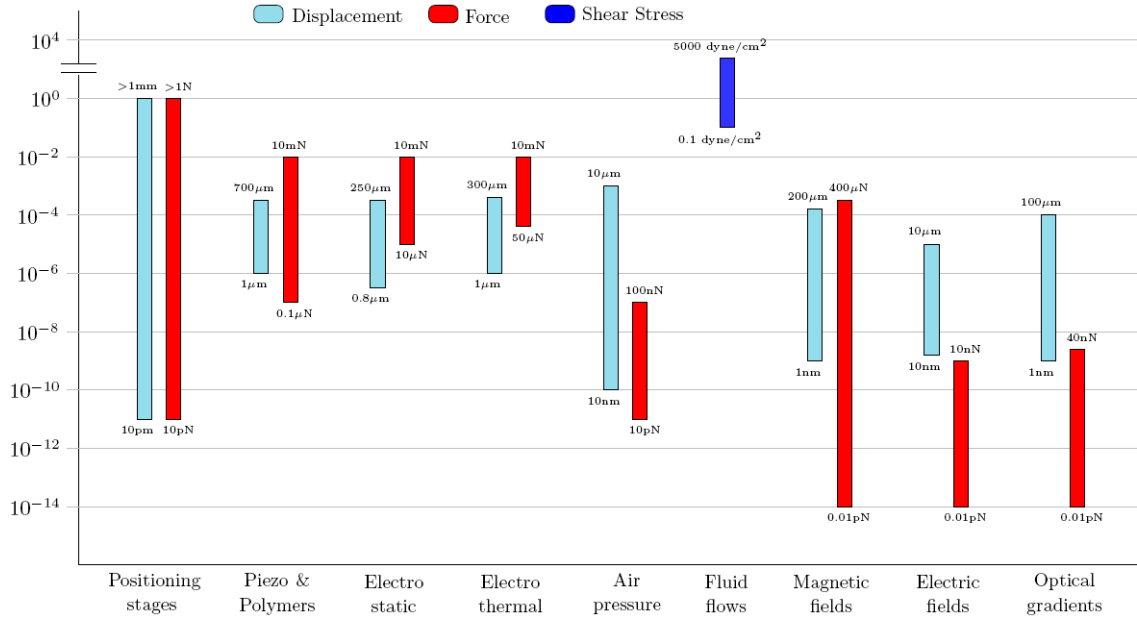


Figure 17: Bar graph evaluating the inherent performances of different actuation means that have been (or might be) found in MEMS for stressing living cells. Values reported here are not limited to the microdevices of this paper, but also take consideration of overall orders of magnitude found in several references (see text for further details)

(Bussolari et al., 1982; Usami et al., 1993; Malek et al., 1995; Cao et al., 1997; Blackman et al., 2000; Dong and Lei, 2000; Hsiai et al., 2002). For magnetic fields, displacement and force amplitudes have been averaged from the analysis of several magnetic tweezers (MT) setups (Evans et al., 1995; Bausch et al., 1998; Simson et al., 1998; Alenghat et al., 2000; Huang et al., 2005; Garcia-Webb et al., 2007; Kollmannsberger and Fabry, 2007; Reed et al., 2008; Spero et al., 2008). Data for electric fields have been fixed according to (Engelhardt and Sackmann, 1988; Zimmermann et al., 2000; Zhang and Liu, 2008). Finally, information about optical gradients has been collected from several optical tweezers (OT) based assays (Hénon et al., 1999; Sleep et al., 1999; Dao et al., 2003;

434 Lim et al., 2004; Li et al., 2009).

435 In Fig. 17, positioning stages appear as the most advantageous mean to ac-
436 tuate MEMS. Indeed, they offer the versatility to combine large travel and force
437 ranges along with very high resolutions. Furthermore, and since they are com-
438 mercially available, they do not involve complex fabrication processes, which
439 greatly simplify their implementation. It is indisputable that displacement and
440 force are parameters of high relevance. Nevertheless, further comparison is re-
441 quired since these features are not sufficient to fairly assess a set of actuation
442 means. Hereafter, some additional specifications are discussed.

443 6.2. *Notion of size and functional density*

At the microscale, the volume of an actuator is a parameter that should not be ignored. Since many of the MEMS actuators presented in this paper can be scaled to different dimensions, an evaluative parameter able to neutralize those variations should be introduced in order to objectively compare different types of actuators. Such a parameter has for instance been proposed in (Carlen and Mastrangelo, 2002):

$$P_a = \frac{F_a \epsilon_a}{V_a} \quad (11)$$

444 where F_a , ϵ_a and V_a are the actuating force, the maximum displacement and
445 the total volume of the actuator considered. By definition, P_a represents the
446 *functional density* (expressed in J/m^3).

447 In the specific context of cell stimulation, one could try to relate P_a to the
448 number of cells that can be actuated by a single actuator. As discussed in Sec-
449 tion 4.2.2, ideal microactuators could independently target a large number of
450 isolated cells, in a minimal volume. Unfortunately, trying to express P_a in such

451 a way for various types of actuation principles is not an easy task. This is espe-
452 cially true in the case of contact-based approaches (i.e., cells are directly touched
453 by the actuator's tip), where the quantity of cells that can be targeted directly de-
454 pends on the type of end effector used. Therefore, a given translation stage could
455 be indifferently linked to a single microcantilever or a matrix encompassing tens
456 of cantilevers, such as the one reported in (Polesel-Maris et al., 2007).

457 Although we are aware of the fact that Equation (11) fails to take into account
458 the number of samples that can be actuated by a given actuation mean, P_a re-
459 mains a valuable parameter to consider in our context (as for all types of MEMS).
460 For instance, it allows one to confirm that the important volume of a commercial
461 positioning stage will actually drastically limit its functional density. Presently,
462 MEMS conceived for high throughput cell screening do not primarily aim at
463 providing autonomous and portable devices. However, the low functional den-
464 sity offered by actuators such as positioning stages might limit further progress
465 in the development of future MEMS for cell mechanics. Conversely, *on-chip*
466 microactuators (e.g., electrothermal, electrostatic actuators) showing high func-
467 tional density may unlock some of the technological gaps currently encountered.

468 6.3. *Notion of biocompatibility*

469 Actuators intended to mechanically stimulate biological cells must deal with
470 additional constraints. Thereby, it appears essential to conserve cells in specific
471 solutions during manipulation. Indeed, cell medium allows the continuous deliv-
472 ery of vital nutrients in order to maintain cells alive. Meanwhile, the performance
473 validated in ambient conditions for some actuators may be significantly altered
474 in the presence of liquids.

475 This is the case for electrostatic comb drives, such as the one in Fig. 6. Due

476 to the hydrophobic nature of the silicon-water interface, intricate phenomena
477 such as air trapping between the comb drive teeth and the MEMS ground plane
478 may arise. Furthermore, the enhanced electrical conductivity of liquids usually
479 reduce their initial stroke.

480 Likewise, electrothermal microactuators also cope with challenging phenom-
481 ena when they are plunged in a liquid environment. For instance, Zhang et al.
482 (2008) underlined the fact that continuous power supply of the device shown in
483 Fig. 5 proves to be unsuitable for underwater operation due to *electrolysis*. Al-
484 though alternating voltages allowed the authors to operate their actuator in elec-
485 trolytic solution, its initial travel range of $9\mu\text{m}$ measured in air was restricted
486 to $4\mu\text{m}$ in liquids. An additional feature of electrothermal actuators relates to
487 the high temperature that they can reach during operation. Since cells are par-
488 ticularly sensitive to temperature fluctuations, high temperatures may potentially
489 cause irreversible damages. Special precautions should hence be taken accord-
490 ingly.

491 This remark might be extended to all types of contact-based actuation means.
492 For instance, in (Boukallel et al., 2009), the authors avoid the use of conventional
493 cantilevers with sharp tip (i.e., such as the ones used for conventional AFM),
494 since the latter could cause damage to external lipid biomembranes during the
495 loading of cells. Regardless of the shape of the mechanical extremity used, con-
496 tamination may occur once the tool touches the cell. Therefore, the tips should
497 be properly cleaned before each new experiment. This additional laborious step
498 may however prevent repetitive analysis.

499 Non-contact actuation techniques would allow to circumvent such a restric-
500 tion. For example, electric fields generated by microfield cages such as the one
501 presented in Fig.2 stretch cells without touching them. However, electric fields

502 can directly affect cells under test (Voldman, 2006). Although no direct contact
503 occurs during stimulation, electric fields cause power dissipation in the form of
504 Joules heating in a conductive medium. Therefore, and as in the case of elec-
505 trothermal actuators, the usage of electric fields requires to monitor changes in
506 temperature that can affect the phenotype of cells.

507 Alternatively, suspended cells can also be stretched without contact with op-
508 tical gradients. Nonetheless, it is admitted that highly concentrated laser beams
509 used in conventional optical tweezers (OT) may be hazardous for cells (Knig
510 et al., 1996; Liang et al., 1996; Neuman et al., 1999; Peterman et al., 2003). In-
511 troduced in Section 2.3, OS made of two optical fibers avoids this problem by
512 reducing the light intensity transmitted to the cell of interest. Indeed, divergent
513 laser beams that stretch the cell are necessarily unfocused, limiting the risk of
514 radiation damage. Consequently, high power lasers can be used without damag-
515 ing the cell. Unfortunately, to date, OS were only proven to be suitable for the
516 stimulation of cells showing a high degree of symmetry and a uniform optical
517 density.

518 Comparatively, MT (see Fig.1) are nowadays considered safe for cells. In-
519 deed, magnetic fields do not significantly disturb or affect the cell response upon
520 short times of exposure required for the application of a mechanical stimulus.
521 Despite this appealing advantage, several restrictions are usually associated with
522 these types of configurations. First, if MT offer the possibility to remotely control
523 magnetic microbeads locally attached to a cell membrane, the magnetic forces
524 applied on the microbeads strongly depends on the beads' size. Meanwhile, it
525 may be difficult to avoid size variations from bead to bead in experimental con-
526 ditions. Likewise, material properties of the beads used (e.g., magnetic moment)
527 cannot be easily controlled and may hence influence the amount of force gener-

ated by a given surrounding magnetic field upon the microparticles. Moreover, the adhesion procedure of the beads remains an unpredictable process. By definition the position of the binding sites as well as the number of magnetic particles adhering to a cell membrane cannot be accurately defined. Accordingly, formation of bead aggregates may appear. Additionally, since bead immersion is unpredictable, the force distribution around adhesion sites can actually be highly heterogeneous.

According to our comparative analysis, no actuation mean may be clearly considered as ideal. Indeed, each actuation method have its own strengths and weaknesses. Accordingly, selection of an appropriate actuator appears mostly possible based on a *trade-off* related to the type of cell investigations that have to be carried out. To sum up this complexity, we propose two charts in Fig.18 for further evaluation between contact and non-contact actuation types. In these charts, desired aspects of key properties required in the specific context of cell stimulation are reported at the extremity of each axis. In consequence, pentagons covering larger surfaces should theoretically represent most appropriate techniques. We however highlight the fact that these charts incorporate criteria that are difficult to objectively estimate. For instance, scientific evidences allowing to quantitatively evaluate the risk of side effects caused by a given actuation technique remain complex to collect. Therefore, criteria reported have been qualitatively ranked based on authors' personal opinions. Nevertheless, and despite their qualitative nature, we believe that these factors remain relevant and should absolutely be considered before selection of an actuation technique.

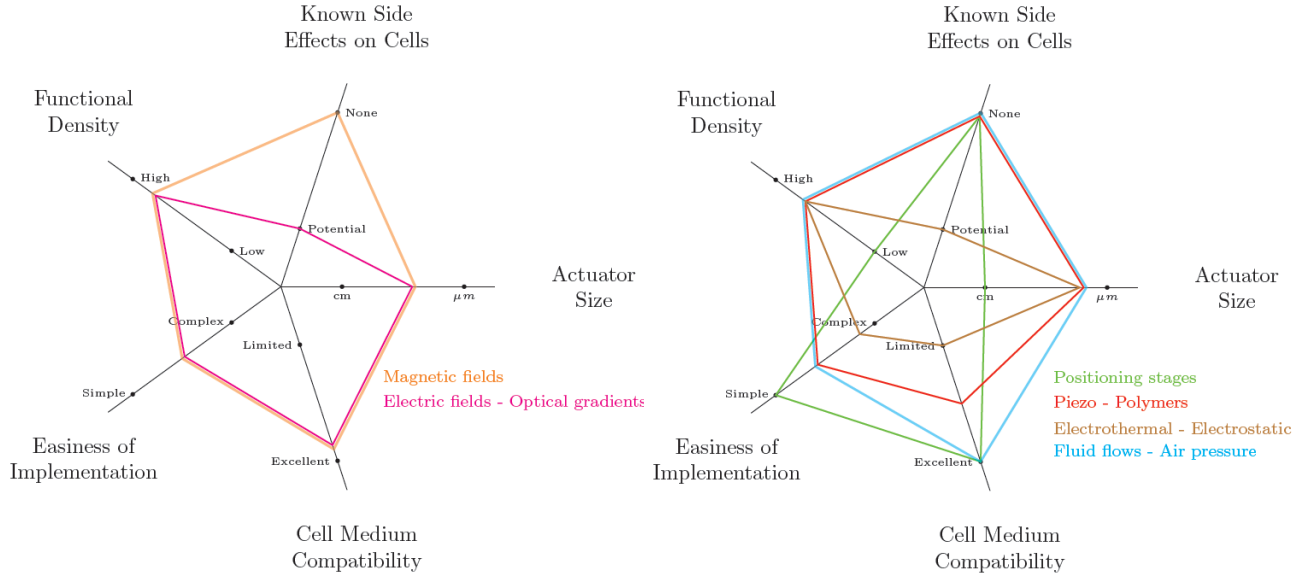


Figure 18: Additional criteria considered for further comparison of the actuation means found in MEMS dedicated to cell stimulation. *Functional Density* corresponds to P_a as defined in Equation (11). It complements the notion of *Actuator Size* by relating the volume with the stress control accuracy that can be achieved by a given actuation technique. *Cell Medium Compatibility* refers the capability of each technique to operate in liquids. From a physical principle, the criterion *Easiness of Implementation* tries to consider the complexity and numbers of processes needed to obtain a functional actuator. Techniques that are known to induce effects on cell phenotype are distinguished in the branch *Known Side Effects on Cells*.

7. Concluding remarks

This paper reports the majority of actuation means currently used in MEMS for the mechanical stimulation of living cells. Additionally, we classify actuation means as a function relative to the amount of cells that they could potentially target. This allowed us to realize that the stimulation of single cells has already been largely addressed. Indeed, many different actuation means have already allowed to accurately stress isolated cells. However, a recent trend aiming at stimulating large amounts of cells emanates from the literature. This trend is mainly justified

559 by the fact that, beyond the technological breakthroughs discovered at the single
560 cell level, new applications require large amounts of isolated cells to perform
561 statistical analyses and result in a better understanding of the cell behavior.

562 To date, parallel and serial cell stimulations remain commonly used by the re-
563 search community. We discussed both approaches, and evaluated different types
564 of actuators. It is interesting to notice that relatively high stimulation rates were
565 achieved using the serial approach which involves optical gradients or electric
566 fields combined with microfluidic channels. However, such configurations are
567 efficient with restrictive types of suspended cells. To our knowledge, stimula-
568 tion rate of adherent cells remains low, even though most cells are anchored to
569 the extracellular matrix *in vivo*, and hence might be in a sense considered as
570 physiologically more relevant. As a matter of fact, the *individual* stimulation of
571 adherent cells in a *high throughput* manner remain presently challenging, and
572 still need to be further addressed.

573 **8. Acknowledgments**

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576 **9. Conflict of Interest Statement**

577 As a condition for publication and/or participation in the reviewing process,
578 we, Denis Desmaële, Mehdi Boukallel and Stephane Regnier, declare that we
579 have no proprietary, financial, professional or other personal interest of any na-
580 ture or kind in any product, service and/or company that could be construed as
581 influencing the position presented in this manuscript.

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